

July 22, 1958

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Screw-Worm Research and Eradication

New Developments

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1. Research at Kerrville, Texas

A. Field tests with sprays and wound dressings

The promising results obtained last summer with Bayer 21/199 and reported at last year's meeting were confirmed this spring in Texas field tests.

Graham, Wrich and Moore of the Kerrville laboratory treated three month's old calves near Del Rio, Texas at the time of castration, dehorning and branding. Of 48 calves treated with Smear 62 (the rancher's standard remedy), 18 became infested within 3 - 14 days. A suspension of 0.5% Bayer 21/199 was swabbed over the wounds and adjacent haired areas to treat 270 calves and 7 of these became infested. A spray of 0.25% Bayer 21/199 applied to wet the whole body was used on 549 calves and only 10 of these developed screw-worms. Further, all animals were completely protected until 14 days after spraying. To summarize -- infestation rates for the various treatments were: Smear 62 - 37%, Swabbing 0.5%, Bayer 21/199 - 2.6%, Spraying 0.25%, Bayer 21/199 - 1.8%

In another test near Del Rio, smaller groups of dehorned calves were treated with a variety of test materials. Poorest results were with Smear 62 which protected over a range of 4 - 12 days; cattle treated with EQ-335 were protected for times ranging from 4 - 19 days. This experiment gave the least protection time yet recorded for 0.25% Bayer 21/199 (CORAL). One of 8 calves became infested 8 days after treatment. A spray of 0.5% protected for a minimum of 21 days. Dow ET-14 (Korlan) sprayed at 1.0%

concentration protected for a range of 13 - 28 days. Smears containing up to 3% of Dow ET-14 or Bayer 21/199 were less effective than the sprays.

Another test near Uvalde, Texas indicated the protection of sprays under conditions of heavy rainfall. Two days after treating dehorned steers, there was 0.5 inches of rain, on the fourth and fifth days 5.5 inches and on the tenth day 2.1 inches. Of 71 cattle smeared with EQ-335, 14 became infested - some as early as 3 days after treatment. Of 61 sprayed with 1.0% Korlan, 10 became infested and 4 of those cases were found at examination 8 days after treatment. Of 61 sprayed with 0.5 % CORAL, only 4 became infested and the earliest case was at the 8 day examination.

B. Toxicity of Bayer 21/199 to livestock.

1. Tests with sheep and goats

Last year entomologists of the Kerrville laboratory sprayed about six thousand newly shorn sheep and goats with Bayer 21/199 to compare concentrations of 0.25 and 0.5 percent. No animals were visibly affected. This winter Dr. Peterson, of the Animal Disease & Parasite Division's Albuquerque laboratory, reported that the concentration of 0.5 percent was toxic to sheep in long fleece. Dr. Radeleff, cooperating Animal Disease and Parasite Research Division veterinarian at Kerrville, then followed up with extensive tests on sheep and goats in full fleece and found that both species of animals were more susceptible to poisoning when their long fleece retained greater quantities of spray.

In order to be sure of a safe treatment for animals in long fleece and to provide a greater margin of safety for treatment of those newly shorn, we recommend a concentration of 0.25% for sheep and goats.

concentration protected for a range of 13 - 28 days. Greater protection up to 38 of low 37-44 of Bayer 21/199 was less effective than the above. Another dose near Uvalde, Texas indicated the protection of groups under conditions of heavy rainfall. Two days after treating sheep, there was 0.5 inches of rain, on the fourth and fifth days 2.5 inches and on the tenth day 2.1 inches. Of 71 cattle treated with 20-55, 14 became infested - none as early as 3 days after treatment. Of 61 sprayed with 1.05 Korian, 10 became infested and 4 of these cases were found at examination 3 days after treatment. Of 61 sprayed with 0.5 Korian, only 4 became infested and the earliest case was at the 3 day examination.

B. Toxicity of Bayer 21/199 to livestock.

1. Tests with sheep and goats

Last year entomologists of the Kerrville laboratory sprayed about six thousand newly shorn sheep and goats with Bayer 21/199 to control concentrations of 0.5 and 0.5 percent. No animals were visibly affected. This winter Dr. Peterson, of the Animal Disease & Parasite Division's diagnostic laboratory, reported that the concentration of 0.5 percent was toxic to sheep in long fleece. Dr. Hahnel, cooperator with the Animal Disease and Parasite Research Division veterinarian at Kerrville, then followed up with extensive tests on sheep and goats in long fleece and found that both species of animals were more susceptible to poisoning when their long fleeces retained greater quantities of spray. In order to be sure of a safe treatment for animals in long fleeces and to provide a greater margin of safety for treatment of those newly shorn, we repeated a concentration of 0.5% for sheep and goats.

We continue to recommend 0.5 percent sprays for cattle more than 6 months old because of the superior residual protection afforded in the higher concentration.

C. Combination treatments with Bayer 21/199 and chlorinated hydrocarbon insecticides.

Cattle shipped within the State of Florida are dipped in a suspension of 0.5% DDT plus 0.03% gamma BHC. Animals moving north of Ocala must also be sprayed with Bayer 21/199 in order to pass the quarantine line. This brought up the question of whether the combination of phosphorus and chlorinated hydrocarbon insecticides might be toxic. The State of Florida may change its tick dip from DDT-BHC to 0.5% toxaphene emulsion. Therefore, the combination with toxaphene had to be considered also.

The Animal Disease Eradication Division purchased 20 baby dairy calves (1 - 2 weeks old) for Dr. Radefeld to treat and observe at Kerrville. First, all 20 were sprayed, each with one gallon of 0.5% of 21/199. Then, after 4 hours, when the animals were thoroughly dry, 10 were sprayed with 1.0% DDT plus 0.06% gamma BHC and 10 were sprayed with 0.75% toxaphene.

Previous research had shown that baby calves were intoxicated by 0.06% gamma BHC, 0.75% toxaphene or 0.5% 21/199. Therefore, any potentiation or even cumulative toxicity from the combination treatments should have caused severe poisoning or death of several of the animals. This did not occur. About half of the calves in each group salivated excessively (a symptom of mild 21/199 poisoning) and one calf in each group suffered convulsions (a symptom of chlorinated hydrocarbon poisoning). Since these reactions were no greater than would be expected from any of the sprays applied alone, it appears that there is no special hazard from the double

no further to treatment 0.5 percent spray for cattle more than 6 months old because of the superior residual protection afforded in the higher concentration.

C. Combination treatment with spray 21/100 and chlorinated hydrocarbon for mosquitoes.

Cattle shipped within the State of Florida was dipped in a suspension of 0.5% DDT plus 0.05% gamma HCH. Animals moving north of Ocala must also be sprayed with spray 21/100 in order to pass the quarantine line. This brought up the question of whether the combination of phosphorus and chlorinated hydrocarbon insecticides might be better. The State of Florida say change the tick dip from DDT-HCH to 0.5% phosphorus emulsion. Therefore, the combination with phosphorus had to be considered also.

The Animal Disease Investigation Division purchased 30 half duty cans (1 - 2 weeks old) for Dr. Radloff to treat and observe at Keweenaw. First, all 30 were sprayed, each with one gallon of 0.5% of 21/100. Then after 4 hours, when the animals were thoroughly dry, 10 were sprayed with 1.0% DDT plus 0.05% gamma HCH and 10 were sprayed with 0.5% phosphorus.

Provision treatment had shown that half calves were infested by 0.05% gamma HCH, 0.75% phosphorus or 0.5% 21/100. Therefore, any combination or even cumulative toxicity from the combination treatments should have caused severe poisoning or death of several of the animals. This did not occur. About half of the calves in each group exhibited excessively (a symptom of mild 21/100 poisoning) and one calf in each group suffered convulsions (a symptom of chlorinated hydrocarbon poisoning). Since these reactions were no greater than would be expected from any of the sprays applied alone, it appears that there is no special hazard from the double

treatment given Florida cattle passing the quarantine line.

2. Methods Development in the Eradication Program

A. Dye-marking of released flies

To supplement the findings of screw-worm scouts, who work with livestock growers in every county in Florida gathering information on case incidence and collecting samples of larvae and eggs from infested animals, more than 400 liver-baited traps are used to catch flies. The number of screw-worm flies caught in traps serves as an index of screw-worm activity in an area prior to the release of sterilized flies. If the traps were to be useful after flies were released in the area, it was necessary to develop some means of marking the released flies. Labelling released flies with radioactive phosphorus was satisfactory in our earlier research, but it was considered impractical for the large-scale release of the eradication program.

Public Health Service workers had reported on a technique for labelling house flies by dusting the adults with oil soluble dyes prior to release in fly migration studies. The dyed flies could be detected by a simple color test.

Cross and Richards of the Methods Development Unit have expanded this lead to develop a practical procedure for marking the sterilized flies. Two liters of sterilized pupae are poured into a plastic bag soon after irradiation. These 18,000 insects are marked by adding to the bag 125 mg. of granular dye. The bag is shaken gently for a couple of minutes to distribute the dye over the puparia. The pupae are then measured into the release boxes. Two days later, when the flies emerge, they crawl about within the box and remove enough dye from the pupal cases so that each fly is marked.

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The dye is not directly visible on the flies, but it is easy to detect. Each screw-worm fly is placed in a small glass vial and washed with a few drops of acetone. Then a small strip of filter paper is placed in the vial so that one end is in the acetone and the other end projects above the lip of the vial. The solvent creeps up the paper and evaporates at the lip of the vial. If the fly is marked, the dye is deposited in a visible line at the place where the paper projects beyond the glass. The vials are set up in racks to evaporate, and the readings are made at the technician's convenience; so it only requires about a minute per fly to do this test.

Laboratory tests with caged flies have shown that every fly that emerges and remains in the release carton for even a few minutes is marked for life. Released flies have been captured in the field marked with a color that had been last used a month before. The main difficulty, at present, is that release schedules sometimes require that flies be released before adult emergence is complete. Checks on fly releases by the Methods Development group have shown that emergence has averaged 94% complete prior to release. These flies are marked, but the 6% remaining pupae that are scattered when the boxes open cannot produce marked flies. It is anticipated that when the assembly line schedule at Sebring operations become effective, there will be an 8-hour instead of a 12-hour spread in ages of pupae at time of packaging and this should permit practically 100 percent marking of flies.

The dyes have been obtained from the American Cyanamid Company. Calco Oil Red and Calco Oil Blue are two colors now being used. Calco Oil Green also marks flies distinctively, but the Company could not be

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Laboratory tests with aged flies have shown that every fly that emerges and remains in the release carton for even a few minutes is marked for life. Released flies have been captured in the field marked with a color that had been used a month before. The main difficulty at present, is that release schedules sometimes require that flies be released before adult emergence is complete. Checks on fly releases by the Method Development Group have shown that emergence has averaged 94% one-plate prior to release. These flies are marked, but the 6% remaining pupae that are recaptured when the boxes open cannot produce marked flies. It is anticipated that when the assembly line schedule is being operated more effectively, there will be an 8-hour instead of a 12-hour period in ages of pupae in time of packaging and this should permit practically 100 percent marking of flies.

The dyes have been obtained from the American Cyanamid Company. Calco Oil Red and Calco Oil Blue are two colors now being used. Calco Oil Green also marks flies satisfactorily, but the Company could not be

sure that this color is safe and nonirritating to the fly packagers, and the release operators who cannot avoid getting some of the material on their skin. Yellow and orange dyes cannot be used on screw-worm flies because acetone extracts similar colors from unmarked flies, and there is not sufficient contrast for reading the paper chromatograms.

The use of red and blue dyes during alternate months in an area enables us to estimate how long flies survive after release. The continued use of red dye on flies released over one area in Florida and blue dye over another area will enable us to study the migration of flies from the areas of release.

B. Larva-rearing procedures

In the research that preceded the eradication program, it was indicated that 100,000 newly hatched larvae (from 5 grams of eggs) could be started in a pan and transferred to a rearing vat with an expected yield of about 50,000 pupae. When it became urgent to produce the maximum number of sterilized flies from the Orlando facilities while Sebring was under construction, the Methods Development groups performed tests to establish procedures for rearing more larvae per vat since no more vats could be crowded into the Bithlo laboratory. A. J. Graham found that by starting larvae from 4 grams eggs in a pan, he could transfer two pans of day-old larvae to a vat, putting one pan on each side. The larvae feed towards the middle of the vat and become mixed into a single culture for the remaining three days of growth. This new technique did not increase the rearing capacity of Bithlo fully 60% as might be inferred from the utilization of 8 grams instead of 5 grams of eggs per vat because crowded larvae did not do quite as well.

sure that this color is safe and nonirritating to the fly pupae, and the release operators who cannot avoid getting some of the material on their skin. Yellow and orange dyes cannot be used on carry-over flies because acetone extracts similar colors from unwanted flies, and there is not sufficient contrast for reading the paper chromatograms.

The use of red and blue dyes during alternate months in an area enabled us to estimate how long flies survive after release. The estimated use of red dye on flies released over one area in Florida and blue dye over another area will enable us to study the migration of flies from the areas of release.

B. Larva-rearing procedures

In the research that preceded the eradication program, it was indicated that 100,000 newly hatched larvae (from 5 grams of eggs) could be started in a pan and transferred to a rearing vat with an expected yield of about 20,000 pupae. When it became urgent to produce the maximum number of sterilized flies from the Orlando facilities while Sabal was under construction, the Methods Development Group performed tests to establish procedures for rearing more larvae per vat since no more vats could be crowded into the Ethio Laboratory. A. J. Graham found that by starting larvae from 4 grams eggs in a pan, he could transfer two pans of day-old larvae to a vat, putting one pan on each side. The larvae feed towards the middle of the vat and become mixed into a single culture for the remaining three days of growth. This new technique did not increase the rearing capacity of Ethio fully 60% as might be inferred from the utilization of 8 grams instead of 5 grams of eggs per vat because crowded larvae did not do quite as well.

However, this development enabled us to use all the eggs produced by the fly colony, and did not limit the rearing to the hatching of 135 grams of eggs for the 27 vats which were all there was room to start each day.

C. Whale meat for larva-rearing

When the eradication program was started, it appeared that ground horse meat and ground beef heart were about equal choices for the main component of the larval diet. Screwworms had been grown on other tissues including ground fish and whale meat, but such larva foods had not been developed for practical large-scale production.

When procurement of supplies was started for the eradication program, it was found that horse-meat supplies were not adequate, and that ground heart was much more expensive than anticipated. Two sources of inexpensive whale meat were located and thousand-pound samples were purchased for testing.

Norwegian whale meat from an Eastern supplier did not work well in practical trials, but Pacific whale meat from California was more promising.

Graham and Cross found that young larvae were particularly sensitive in their food requirements and grew much better on beef or horsemeat. The older larvae tolerated the whale meat and larvae that finished their growth on whale meat were as good as those grown on horse meat or beef. As a result, three different media are used to rear the larvae:

1. The newly hatched larvae are reared in pans for 24 hours on a starting medium composed of: horse meat - 47%, bovine plasma - 26.5%, water - 26.3% and formalin (preservative) 0.2%.

2. On the second and third days of growth, the larvae are reared in vats on: horse meat - 54%, bovine blood - 15%, water 29.8%, and

However, this development enabled us to use all the eggs produced by the fly colony, and did not limit the rearing to the hatching of 155 grams of eggs for the 27 vials which were all there was room to store each day.

C. Whale meat for larva-rearing

When the stratification program was started, it appeared that ground horse meat and ground beef heart were about equal choices for the main component of the larval diet. Some workers had been given on other slimes including ground fish and whale meat, but much larva food had not been developed for practical large-scale production.

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As a result, three different media are used to rear the larvae:

1. The newly hatched larvae are reared in pans for 24 hours on a starting medium composed of: horse meat - 47%, bovine plasma - 36.3%, water - 36.3% and formalin (preservative) 0.2%.
2. On the second and third days of growth, the larvae are reared in vials on: horse meat - 54%, bovine blood - 15%, water 29.6%, and

formalin - 0.2%

3. To finish their growth, the larvae are fed on the fourth and fifth days: whale meat - 58%, bovine blood - 14%, water - 27.8%, and formalin 0.2%.

The total requirements to rear a vat of 50,000 - 75,000 larvae are:

Horse meat - 40 pounds
Whale meat - 80 pounds
Plasma - 500 cc
Blood - 3.4 gallons
Water - 6.8 gallons
Formalin - 240 cc

Since horse meat costs about 25 cents per pound and whale meat 13 cents, there is an important saving. Horsemeat alone for 50,000 larvae would cost \$30.00. By feeding the combination, the horse meat costs \$10.00, and the whale meat \$10.40. This represents a saving of \$9.60 per vat or \$9,600 per week when the Sebring plant is in full production.

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Form 10 - 0.32

3. To limit their growth, the larvae are fed on

12th days: whole meal - 25%, feeding blood - 1%,

Form 10 - 0.32

The total requirements to rear a vat of 20,000 - 75,000

Flour meal - 40 pounds

Whole meal - 80 pounds

Flour - 200 lbs

Blood - 2.5 gallons

Water - 6.5 gallons

Form 10 - 0.32

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and the whole meal \$10.40. This represents a saving of 29.60 per vat or

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